Interaction of Salt, pH, and Temperature on the Growth and Survival of Salmonellae in Ground Pork

JOHN A. ALFORD AND SAMUEL A. PALUMBO

Eastern Utilization Research and Development Division, Agricultural Research Service,
United States Department of Agriculture, Beltsville, Maryland 20705

The interaction of temperature, pH, and NaCl concentration on the growth and survival of several strains of salmonellae has been determined in broth and ground pork. Growth of 23 strains occurred in broth at 30 C over a wide range of pH-NaCl combinations; at 10 C, growth was limited to only a few combinations. Cultures which would not grow at 10 C because of the pH-NaCl effect survived for long periods, however. In contrast, cultures which would not grow at 30 C remained viable for only a short time. Results in fresh ground pork were in close agreement with the broth studies. Salmonellae would not grow in ground pork stored at 4 C but would grow in pork containing 3.5% salt stored at 10 C. Salmonellae grew competitively with the natural background flora at 10 C even when the salmonellae constituted less than 5% of the initial flora, and the background flora would grow at a lower temperature than the salmonellae. The data show that, whereas decreasing temperatures increase the inhibitory effects of pH and NaCl, they decrease the lethal effects.

The ability of salmonellae to survive in food products held at low temperatures has been well established (3, 5, 15, 16), but the relationships of pH, NaCl concentration, and temperature on the survival of a wide variety of strains are less clear. Data on growth, as distinct from survival, of salmoneallae in meats and meat products in competition with the normal flora and the effect of pH and NaCl on this growth at low temperatures are almost totally lacking (7, 8). In fact, investigations on the growth of salmonellae in other food products usually have involved sterile slurries (2, 6, 8, 10) held at low temperatures, or mixtures of salmonellae and other contaminants incubated at 25 to 37 C (6, 11), or products in which NaCl concentration and pH were not considered (10).

This investigation, therefore, had a twofold purpose: first, to determine any differences in the effects of NaCl, pH, and temperature on the growth and survival of a large number of salmonella strains; second, and of more direct concern, to determine the effect of these parameters on the growth of selected salmonellae in ground pork in competition with its natural flora.

MATERIALS AND METHODS

Organisms. The test organisms used and their sources are listed in Table 1. The organisms were chosen as representing species likely to be found as food contaminants.

Medium for broth studies. The test medium used for the broth studies was a modified Trypticase Soy Broth (BBL) of the following composition (final concentration in grams per liter): TSB, 30; K_2HPO_4, 6; KH_2PO_4, 3; $NaNO_3$, 0.1370 (1,000 μg/g NO_3^-); and $NaNO_2$, 0.0685 (500 μg/g NO_2^-). Broths were prepared containing 2, 5, and 8% NaCl, final concentration. Each salt concentration was divided into three portions; these were adjusted to pH 6.5, 5.8, and 5.0 with 10% H_3PO_4. All broth tubes were sterilized by autoclaving.

Growth and survival in broth studies. Triplicate tubes of each of the 9 NaCl-pH combinations were inoculated with 0.1 ml of a 24-hr culture and incubated at 10 C ($\pm$1), 20 C ($\pm$1), and 29 C ($\pm$2), respectively. Growth was assessed on the basis of visible turbidity. At intervals, 1-ml samples of cultures not showing growth were added to tubes containing 9 ml of tempered (45 C) 1.1 $\times$ glucose-purple agar (Difco). This dilution eliminated the inhibitory effects of NaCl and pH, and survival was indicated by acid-gas production within 72 hr at 29 C ($\pm$2).

Effect of type of acid in broth studies. To determine

TABLE 1. *Identity and sources of Salmonella cultures used in this investigation*

Designation	Identity	Designation	Identity
A	*Salmonella senftenberg* 775W[a]	N	*S. derby* AM #21[b]
B	*S. senftenberg* S8[a]	O	*S. cholerae-suis* AM #34[b]
C	*S. blockley* 2004[a]	P	*S. cholerae-suis* var. *kunzendorf* AM #36[b]
D	*Salmonella* sp.[b,c]		
E	*Salmonella* sp.[b,c]	R	*S. thompson* var. *berlin* AM #40[b]
F	*Salmonella* sp.[b,c]	Q	*S. thompson* AM #39[b]
G	*Salmonella* sp.[b,c]		
H	*S. typhimurium* AM #9[b]	R	*S. thompson* var. *berlin* AM #40[b]
I	*S. typhimurium* AM #10[b]	S	*S. oranienburg* AM #42[b]
J	*S. typhimurium* AM #11[b]	T	*S. enteriditis* AM #64[b]
K	*S. typhimurium* #12[b]	U	*S. anatum* AM #78[b]
L	*S. chester* AM #17[b]	V	*S. meleagridis* AM #109[b]
M	*S. derby* AM #20[b]	W	*S. bredeney* AM #112[b]
		X	*S. tennessee*[d]
		Y	*S. typhimurium*[d]

[a] From Western Utilization Research and Development Division, USDA, Albany, Calif.

[b] From Alice Moran, Consumer and Marketing Service, USDA, Beltsville, Md.

[c] Isolated from salted hog casing.

[d] From Communicable Disease Center, Atlanta, Ga.

whether different organic acids would affect growth and survival, one lot of broth containing 3% NaCl was divided into three portions and adjusted to pH 5.0 with 0.5% (w/v) lactic, acetic, and phosphoric acids, respectively. After inoculation, these tubes were incubated at 10 C and observed for growth and survival as described above.

Ground pork studies. Ground pork was prepared from fresh hams that had been frozen and held at −34 C until needed. After thawing, the lean and fat portions were separated aseptically, cut into 2.5-cm (1 inch) cubes, and combined to give a mixture of approximately 20% added fat. The meat cubes, together with proper amounts of NaCl and cultures, were placed in a sterile jar and mixed slowly (30 rev/min) for 10 min on a Ball Mill (refrigerated). The inoculated meat then was ground once in a sterile grinder ($\frac{3}{16}$-inch die), packaged in 11-g amounts in Whirlpak bags, and stored.

Counting. Total viable cell counts were made by surface plating, onto plates of Tryptic Soy Agar (TSA; Difco), appropriate dilutions of an 11-g sample in 0.1% peptone-water. The plates were incubated at 25 C for 48 hr and then counted.

Enumeration of salmonellae usually was done by plating duplicate samples of dilutions of the sample onto dry plates of Brilliant Green (BG) agar (Difco) and Xylose-Lysine Desoxycholate (XLD) agar (Difco) by the surface plating technique. The plates were incubated at 37 C for 48 hr; this incubation period gave better differentiation between salmonellae and other gram-negative bacteria than did the 24-hr incubation.

The five tube-three dilution most-probable-number method was used when very small numbers of salmonellae were anticipated or when coliforms were encountered as part of the contaminating flora of the ground pork. Selenite-Cystine broth (Difco), incubated at 37 C for 24 hr, was used for enrichment and was followed by streaking onto BG agar plates. Representative colonies from positive plates were picked and verified by inoculating Triple Sugar Iron agar (Difco). The most-probable-number then was determined by use of an appropriate table (1).

Fluorescent-antibody technique. The fluorescent-antibody technique was utilized to verify that colonies counted as salmonellae on BG and XLD agars were actually salmonellae. It also was used as a second confirmatory method of the Selenite-Cystine enrichment tubes from the most-probable-number method. In both instances, fluorescein-tagged polyvalent O antiserum (groups A to H; Sylvana, Milburn, N.J.) was used according to procedures employed at the Communicable Disease Center, Atlanta, Ga. (*personal communication*).

RESULTS

Broth studies. Preliminary experiments indicated that salmonellae would grow in the presence of much higher concentrations of nitrite and nitrate than may be legally added to meats. Therefore, only one level (equal to about twice the permissible nitrite concentration) was included in these experiments.

The combined influence of temperature, NaCl, and pH on the ability of 23 strains of salmonellae (A to W) to initiate growth in broth is shown in Table 2. Whereas at 10 C, growth of 22 of 23 strains was inhibited by a pH of 5.0 and 2% NaCl, at 20 and 30 C, all strains grew well under these conditions. At pH 5.8, the pH more likely to be found in meats and meat products, 5% NaCl was required at 10 C to inhibit growth. Increasing NaCl concentrations caused a slight decrease in survival time at 10 C, whereas, within the range studied, pH had little or no effect.

In an experiment comparing the effects of lactic, acetic, and phosphoric acids as pH adjusters, an analysis of variance of the survival times of the 25 strains (A to Y) indicated that there were no effects attributable to the different acids (at the 95% confidence level). As in the other broth studies, considerable differences were observed in time of survival among the strains of salmonellae.

Ground pork studies. Two storage temperatures,

TABLE 2. *Influence of pH, NaCl concentration, and temperature on growth and survival in broth of 23 strains of Salmonella*

Incubation temp	Growth or days surviving	No. of strains growing or surviving at:								
		pH 5.0			pH 5.8			pH 6.5		
		2% NaCl	5% NaCl	8% NaCl	2% NaCl	5% NaCl	8% NaCl	2% NaCl	5% NaCl	8% NaCl
10 C	Growth	1[a]	0	0	23	1[a]	0	23	1[a]	0
	>84 days	20	10	1		10	7		13	7
	>20, <70	2	13	21		12	16		9	15
	<14			1						1
20 C	Growth	23	3[b]	0	23	23	6	23	23	12
	>30		15	16			11			8
	>4, <30		5	7			6			3
30 C	Growth	22	5		23	23	15	23	23	23
	>14		4				2			
	>2, <7	1	14	20			6			
	<2			3						

[a] *Salmonella senftenberg* S8.
[b] *S. senftenberg* S8, *S. senftenberg* 775 W, *S. bredeney*.

10 and 4 C, were utilized as representing temperatures just above and below the recorded minimum temperature of growth for salmonellae (2, 10). These two temperatures are also representative of poor and average holding conditions found in home and commercial refrigerators. *Salmonella chester, S. derby, S. typhimurium, S. thompson,* and *S. enteritidis,* selected as representative of the more prevalent strains isolated from food, grew well in ground pork at 10 C but not at 4 C. Similar counts were obtained on both BG and XLD agars. When the background count was low ($< 10^3$/g) as compared to the salmonellae count ($\pm 10^4$/g), the TSA counts were predominantly salmonellae and they closely paralleled the BG and XLD counts.

When the salmonellae accounted for 10% or less of the total flora, they were still able to compete at 10 C. The data in Fig. 1 show the growth of three *Salmonella* species in competition with a background flora composed primarily of coliforms. In one instance, *S. enteritidis,* the initial count was less than 200 while the background count was over 5,000. At 4 C, they disappeared slowly while the psychrophilic flora increased steadily. Salmonellae were still detectable, however, when the meat was organoleptically spoiled.

During some of the ground pork studies, the salmonella counting procedures (plating and most-probable-number) were cross-checked by use of the fluorescent-antibody technique. Of 116 positive colonies on BG and XLD agars, 104 or 89.6% were positive by the fluorescent-antibody technique. Of the Selenite-Cystine enrichment
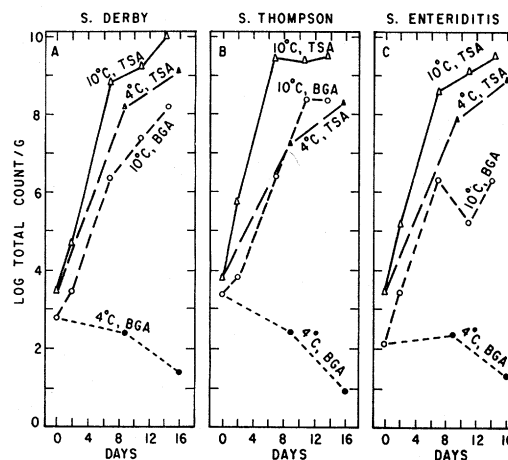

FIG. 1. *Growth of Salmonella spp. and total flora in ground pork with a relatively high background count and containing 2% NaCl.*

tubes examined, all those positive by the fluorescent-antibody technique were subsequently found positive by cultural methods.

When three strains of salmonellae were added, along with either gram-negative rods isolated from an earlier experiment or a diluted slurry of commercial pork sausage, to lots of freshly ground pork containing different levels of NaCl, then stored at 10 C, the salmonellae grew in all four trials in 0 and 2% NaCl. In 3.5% NaCl there was a lag of 4 to 7 days in three of four trials and at least 14 days in the fourth. No in-
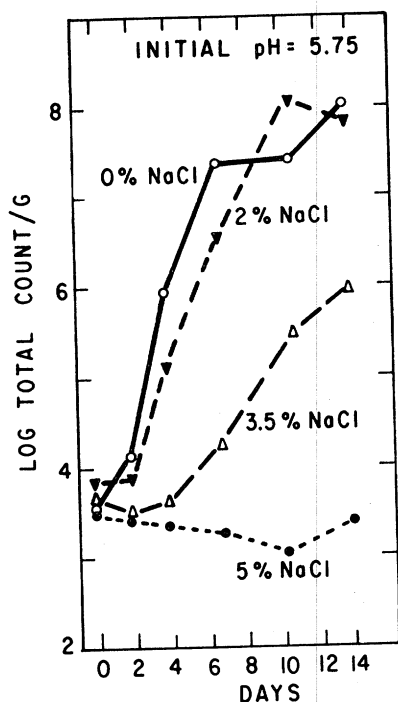
FIG. 2. *Influence of NaCl concentration on growth of salmonellae in ground pork stored at 10 C; counts on BG agar.*

crease in salmonellae occurred in any of the samples containing 5% NaCl for at least 14 days. Although the initial pH of the meat was 5.7 in two of the trials and 6.2 and 6.4 in the other two, no relationship of growth to this narrow pH range could be shown. Figure 2 presents representative data from one of these trials.

DISCUSSION

Although BG and XLD agars are commonly used to isolate salmonellae from meats and meat products, there is little direct evidence about the quantitative accuracy of such procedures. The ability to selectively inhibit salmonellae by temperature allows a study of the ability of these media to enumerate these organisms in the absence and presence of a competing microflora. Had the appearance of false-positives caused by the competing microflora been a problem, then the BG and XLD counts on samples held at 4 C should have increased. Such was not the case. This is further substantiated by the confirmation by fluorescent staining of 90% of the typical BG colonies picked from both 4 C and 10 C experiments. The similarity in growth rates of salmonellae at 10 C, when they were both in a

minority and a majority of the initial flora, suggests that false-negatives were a minor problem too. Thus, we can be reasonably sure that the counts on BG and XLD agars gave a reliable indication of the salmonella count.

The point mentioned above concerning a normal growth rate occurring in the presence of a competing flora appears to contradict reports of other workers (6, 11). In these reports, however, the salmonella-natural flora mixtures were incubated at 35 to 37 C. Except for a specialized product like dried eggs or albumen, it is unlikely that such a temperature would be encountered in commercial practice. Matches and Liston (10) found that salmonellae inoculated onto naturally contaminated English sole and incubated at 8 to 12 C grew competitively. In the ground pork stored at 4 C, the concentration of salmonellae declined slowly, but they were still present when the meat was organoleptically spoiled. Thus, it is unrealistic to suggest that the competing flora in a product requiring refrigeration might render it salmonella-free while still edible.

Ingram and Kitchell (7) recently stated that the influence of temperature on the inhibitory effect of salt (i.e., prevention of growth as distinct from its lethal effect) was uncertain and merited further investigation. The data presented here (Table 2) show that, at a constant pH, the salt concentration required to inhibit salmonellae decreases as the temperature decreases. This is in direct contrast to the higher concentrations required to kill salmonellae as the temperature decreases (4, 14). Studies by Ohye and Christian (12) and Segner, Schmidt, and Boltz (13) on the spores of *Clostridium botulinum* type E have shown a similar decrease in NaCl concentration required to prevent outgrowth as the temperature decreases.

Although the moisture content of the ground pork was not determined directly, it can be calculated from the known fat and protein content to be approximately 65 to 70%. A 3.5% concentration of NaCl added on a weight basis to the meat would give about 5% NaCl in the aqueous phase. Thus, the delayed growth of salmonellae at 10 C in ground pork containing 3.5% NaCl and the inhibition by 5% NaCl in broth at 10 C indicate that this temperature-NaCl concentration is near the limit permitting outgrowth of salmonellae.

The well-established inhibitory effect of decreasing pH, particularly in conjunction with NaCl (7, 8, 12, 13), has been shown again in this work. However, except for fermented sausages, it is unlikely that the pH of any meat or meat

product would have a significant effect on the growth or survival of salmonellae.

The determination of most-probable-number of a food in Selenite-Cystine enrichment medium with confirmation by the fluorescent-antibody technique offers a rapid and reliable method for studying growth of salmonellae in a contaminated food product under a variety of conditions.

A food contaminated with any number of salmonellae is potentially a source of a foodborne outbreak and is, therefore, suspect. Nevertheless, it is recognized that the number of salmonellae ingested is related to the likelihood of an infection developing (9). Thus, the minor changes in pH and salt concentration introduced by the food technologist for their effects on flavor and texture may inhibit or permit growth in marginally stored products.

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